In another preferred embodiment, an *in vivo* process for delivering a polynucleotide to a parenchymal cell of a mammal is described. First, the polynucleotide is inserted into a blood vessel. Then, interior blood flow is externally impeded and the naked DNA is delivered to the parenchymal cell. The polynucleotide may consist of naked DNA, a viral particle/vector, a non-viral vector or may be a blocking polynucleotide for preventing gene expression. The parenchymal cell may consist of a muscle cell, such as a limb (leg or arm) muscle cell.

The process includes externally impeding interior blood flow by externally applying pressure to interior blood vessels such as compressing mammalian skin by applying a tourniquet over the skin. Compressing mammalian skin also includes applying a cuff over the skin such as a sphygmomanometer.

In another preferred embodiment, an *in vivo* process for delivering a polynucleotide to a mammalian cell, consists of inserting the polynucleotide into a blood vessel and applying pressure to the blood vessel. The pressure is applied externally to mammalian skin and the polynucleotide is delivered to the mammalian cell. However, it is important that the full function of the mammal's limbs subsequent to delivery is maintained using this process. The process especially consists of a polynucleotide delivered to non-vascular (not of the smooth muscle cells surrounding a vessel) parenchymal cells.

In yet another preferred embodiment, a device for applying pressure to mammalian skin for *in vivo* delivery of a polynucleotide to a mammalian cell is described. The device consists of a cuff, as defined in this specification, applied to mammalian skin to impede blood flow thereby increasing delivery efficiency of the polynucleotide to the mammalian cell.

In a preferred embodiment it may be preferential to immunosuppress the host receiving the nucleic acid. Immunosuppression can be long term or for a short duration, preferably around the time of nucleic acid delivery. This can be accomplished by treatment with (combinations of) immunosuppresive drugs like cyclosporin A, ProGraf (FK506), corticosteroids, deoxyspergualin, and dexamethason. Other methods include blocking of immune cell activation pathways, for instance by treatment with (or expression of) an antibody directed against CTLA4; redirection of activated immune cells by treatment with (or expression of)

chemokines such as MIP-1a, MCP-1 and RANTES; and treatment with immunotoxins, such as a conjugate between anti-CD3 antibody and diphtheria toxin.

Further objects, features, and advantages of the invention will be apparent from the following detailed description when taken in conjunction with the accompanying drawings.

Brief Description of the Drawings

FIG. 1 Photomicrographs of muscle sections histochemically stained for ß-galactosidase expression. Panel A represents a muscle (pronator teres) with a high level of expression; panel B represents a muscle (abductor pollicis longus) with an average level of expression. Magnification: 160X.

FIG. 2A-2C Expression of ß-galactosidase (light grey) and GFP (white) in rat muscle injected intraarterially at different times with the respective expression pDNAs. Panel A (640X magnification) is a low-power field illustrating that expression of ß-galactosidase and GFP were typically not co-localized. Panels B and C are high power fields (1600X magnification) that show an example of co-localization (B) and separate expression (C

FIG. 3A-3C Muscle sections obtained 5 min (A and B) and 1 h (C) after 50 µg of Rh-pDNA in 10 ml of normal saline were injected within 7 sec into the femoral artery of rat without impeding the outflow (A) or impeding outflow (B and C). Arrows indicate Rh-pDNA between cells and arrowheads indicate pDNA inside myofibers. Magnification: 1260 X

Detailed Description

We have found that an intravascular route of administration allows a polynucleotide to be delivered to a parenchymal cell in a more even distribution than direct parenchymal injections. The efficiency of polynucleotide delivery and expression is increased by increasing the permeability of the tissue's blood vessel. Permeability is increased by one or more of the following: increasing the intravascular hydrostatic (physical) pressure, delivering the injection fluid rapidly (injecting the injection fluid rapidly), using a large injection volume, inhibiting vessel fluid flow, and increasing permeability of the vessel wall. Prior to